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US-PAT-NO: 6090558
DOCUMENT-IDENTIFIER: US 6090558 A

TITLE: DNA typing by mass spectrometry with polymorphic DNA repeat markers

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US-CL-CURRENT: 435/6; 435/91.2, 435/91.5, 436/173

Full Title CIT.1 REV.1 CLS.1 REF.1 DRAW.1

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L6: Entry 1 of 1 File: USPT Jul 18, 2000

DOCUMENT-IDENTIFIER: US 6090558 A
TITLE: DNA typing by mass spectrometry with polymorphic DNA repeat markers

ABPL:
The present invention is related to the fields of genetic mapping and genetic identity detection, including forensic identification and paternity testing. This invention is more specifically directed to the use of mass spectrometry to detect length variation in DNA nucleotide sequence repeats (including variants of common alleles), such as microsatellites and short tandem repeats, and to DNA sequences provided as primers for the analysis of DNA tandem nucleotide repeat polymorphisms at specific loci on specific chromosomes.

BSPR:
The present invention is generally directed to the field of genetic identity detection including forensic identification and paternity testing as well as genetic mapping. The present invention is more specifically directed to the use of mass spectrometry to detect length variations in DNA nucleotide sequence repeats, often referred to as short tandem repeats ("STR"), microsatellite repeats or simple sequence repeats ("SSR"). The invention is also directed to DNA sequences provided for the analysis of STR polymorphisms at specific loci on specific chromosomes.

BSPR:
Many different primers have been designed for various DTNR loci and reported in the literature. These primers anneal to DNA sequences outside the DNA tandem repeat region to produce PCR.TM. products usually in the size range of 100-800 bp. These primers were designed with polyacrylamide gel electrophoretic separation in mind, because DNA separations have traditionally been performed by slab gel or capillary electrophoresis. However, with a mass spectrometry approach to DTNR typing and analysis, examining smaller DNA oligomers is advantageous because the sensitivity of detection and mass resolution are superior with smaller DNA oligomers.

BSPR:
The advantages of using mass spectrometry for characterizing DTNRs include a dramatic increase in both the speed of analysis (a few seconds per sample) and the accuracy of direct mass measurements. In contrast, electrophoretic methods require significantly longer lengths of time (minutes to hours) and can only measure the size of DTNRs as a function of relative mobility to comigrating standards. Gel-based separation systems also suffer from a number of artifacts that reduce the accuracy of size measurements. These mobility artifacts are related to the specific sequences of DNA fragments and the persistence of secondary and tertiary structural elements even under highly denaturing conditions.

BSPR:
The second approach to multiplexing 2 or more DTNR loci on gel-based systems is the use of spectroscopic partitioning. Current state of the art for gel-based systems involves the use of fluorescent dyes as specific spectroscopic markers for different PCR.TM. amplified loci. Different chromophores that emit light at different color wavelengths provide the means for differential detection of two different PCR.TM. products even if they are exactly the same size, thus 2 or

more loci can produce PCR.TM. products with allele size ranges that overlap. For example, Locus A with a green fluorescent tag produces an allele size range from 250 to 300 nucleotides, while Locus B with a red fluorescent tag produces an allele size range of 270 to 330 nucleotides. A scanning, laser-excited fluorescence detection device monitors the wavelength of emissions and assigns different PCR.TM. product sizes, and their corresponding allele values, to their specific loci based on their fluorescent color.

BSPR:
In contrast, mass spectrometry directly detects the molecule preventing the use of optical spectroscopic partitioning as a means for multiplexing. While it is possible to have a limited use of size partitioning with TOF-MS, the limited size range of high-resolution detection by TOF-MS makes it likely that only 2 different loci can be multiplexed and size partitioned. In many cases, it may not be possible to even multiplex 2 loci and maintain a partitioning of the 2 different allele size ranges. Therefore, new methods are needed in order to employ mass spectrometry for the analysis of multiplexed DTNRs.

BSPR:
It is, therefore, a goal of the present invention to provide newly designed PCR.TM. primers which are closer to the repeat regions then have previously been employed providing for the efficient analysis by TOF-MS. Specifically, the invention provides oligonucleotide primers designed to characterize various DTNR markers useful for human identity testing. The primers are for use in PCR.TM. amplification schemes, however, one of skill in the art could, in light of the present disclosure, employ them to generate appropriate size nucleic acid products for TOF-MS analysis using other methods of extending one or more of the disclosed primers. Additionally, these primers and their extension products are suitable for detection by mass spectrometry. Thus, applications of this invention include forensic and paternity testing and genetic mapping studies.

BSPR:
The primer extension products are preferably single-stranded and may be any size that can be adequately resolved by mass spectrometric analysis. Preferably, detected, the final product single-stranded target nucleic acids are less than about 160 or 150 bases in length. More preferably, the extended nucleic acid products are from about 10 to 100 or 120 bases in length. As used in this context, "about" means anywhere from .+-.1 to 20 bases, and all the integers in between, for example, .+-.1, .+-.2, .+-.3, .+-.4, .+-.5, .+-.6, .+-.7, .+-.8, .+-.9, .+-.10, etc. bases.

BSPR:
Examples of some oligonucleotide primers that may be employed for amplifying these loci are listed in SEQ ID NO:1 through SEQ ID NO:103. Preferred oligonucleotide primers that also contain a cleavable phosphorothioate linkage and biotin moiety for immobilization on an avidin, streptavidin solid support are sequences according to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100 and SEQ ID NO:103. These newly designed primers generate nucleic acid extension products which are smaller than those used previously with electrophoresis separation methods. Additionally, these primers may be used in other methods of primer extension known to those of skill in the art.

BSPR:
Another embodiment of this invention encompasses a kit for analyzing alleles of a DTNR locus in a target nucleic acid, having a first strand and a second complementary strand, by mass spectrometry which includes a first primer complementary to the flanking region of a DNA tandem nucleotide repeat region and a second primer complementary to the opposite flanking region of a DNA tandem nucleotide repeat region. Preferred kits of this invention are kits for analyzing the following DTNR loci: CSF1PO, D3S1358, D5S818, D7S820, D8S1179,

D13S317, D16S539, D18S51, D21S11, DYS19, F13A1, FES/FPS, FGA, HPRTB, TH01, TPOX, DYS388, DYS391, DYS392, DYS393, D2S1391, D18S535, D2S1338, D19S433, D6S477, D1S518, D14S306, D22S684, F13B, CD4, D12S391, D10S220 and D7S523.

BSPR:
Another embodiment of this invention encompasses a kit for analyzing alleles of a multiple DTNR loci in a target nucleic acid by mass spectrometry, which includes a plurality of primers complementary to the flanking regions of DNA tandem nucleotide repeat regions. Preferred kits of this invention are kits for analyzing the following DTNR loci: CSF1PO, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, DYS19, F13A1, FES/FPS, FGA, HPRTB, TH01, TPOX, DYS388, DYS391, DYS392, DYS393, D2S1391, D18S535, D2S1338, D19S433, D6S477, D1S518, D14S306, D22S684, F13B, CD4, D12S391, D10S220 and D7S523.

BSPR:
The primers employed with these kits may preferably have cleavable sites, such as a recognition site for a restriction endonuclease, an exonuclease blocking site, or a chemically cleavable site. Preferred chemically cleavable sites encompass modified bases, modified sugars (e.g., ribose), and chemically cleavable groups incorporated into the phosphate backbone, such as dialkoxysilane, 3'-(S)-phosphorothioate, 5'-(S)-phosphorothioate, 3'-(N)-phosphoroamidate, or 5'-(N)-phosphoroamidate linkages. Another preferred embodiment is a kit employing a first primer that is capable of attaching to a solid support.

BSPR:
The present invention also focuses on an improved method of multiplexing the analysis of nucleic acid extension products derived from DNA nucleotide repeat loci. This method differs from known methods of multiplexing DTNR analysis in that mass spectrometry is employed and the range of possible nucleic acid extension products for the multiplexed loci, the allele nucleic acid extension product size ranges, may be specifically chosen to overlap in the mass scale yet be uniquely resolved and detected.

BSPR:
Another embodiment of this invention encompasses a method for multiplexing the detection of more than one amplified DNA tandem nucleotide repeat marker from more than one DNA tandem nucleotide repeat loci including: determining the mass of more than one nucleic acid extension product by mass spectrometry, where the DNA tandem nucleotide repeat loci each comprise a DNA tandem repeat sequence and a flanking sequence and have overlapping allelic mass ranges. Typically, at least one of the target nucleic acid extension products may contain a mass modifying group.

DEPR:
The invention further relates to primers designed to characterize 33 DNA repeat markers useful for human identity testing. Applications include forensic and paternity testing as well as genetic mapping studies. These DTNR markers are useful in PCR.TM. amplification, preferably as pairs of oligonucleotide primers, and in other methods of primer extension may be used as single primers, the extension products of which may be accurately detected by mass spectrometry as they are smaller than those used previously with electrophoresis separation methods.

DEPU:
Kimpton, Oldroyd, Watson, Frazier, Johnson, Millican, Urquhart, Sparkes, Gill, "Validation of highly discriminating multiplex short tandem repeat amplification systems for individual identification," Electrophoresis, 17:1283-1293, 1996.

DEPU:
Braun, A., et al., "Detecting CFTR gene mutations by using primer oligo base extension and mass spectrometry," Clin. Chem. 43:1151-1158 (1997).

DEPU:
Wenz, H.-M., et al., "High-Precision Genotyping by Denaturing Capillary

Electrophoresis," Genome Res. 8:69-80 (1998).

ORPL:
Braun et al., "Detecting CFTR gene mutations by using primer oligo base extension and mass spectrometry," Clin. Chem. 43:1151-1158, 1997.

ORPL:
Kimpton et al., "Validation of highly discriminating multiplex short tandem repeat amplification systems for individual identification," Electrophoresis, 17:1283-1293, 1996.

ORPL:
Lee, et al., Comparison on short tandem repeat (STR) detection using silver, fluorescence and matrix assisted laser desorption ionization time-of-flight mass spectrophotometry (MALDITOF-MS), Proceedings of the Sixth International Symposium on Human Identification, published by Promega Corp., 1995.

ORPL:
Liu et al., "Rapid screening of genetic polymorphisms using buccal cell DNA with detection by matrix-assisted laser desorption/ionization mass spectrometry," Rapid Commun. in Mass Spectrometry, 9:735-743, 1995.

ORPL:
Tang et al., "Detection of 500-nucleotide DNA by laser desorption mass spectrometry," Rapid Commun. in Mass Spectrometry, 8(9):727-730, 1994.

ORPL:
Wenz et al., "High-Precision Genotyping by Denaturing Capillary Electrophoresis," Genome Res. 8:69-80, 1998.